The Lister Institute of Preventive Medicine.

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Dear Dr.Lederberg,

Thank you very much for your letter of 12th March. I will try today to give you some "concrete account of my observations", though I feel it is rather difficult to convey to you in writing, what I see. It may help if I explain my new hypothesis which has been derived from my observations. Yet the evidence is not so complete that I could say, I have proved it. I therefore call it a hypothesis. It is this :

Under various conditions - some of which are probably slightly unfavourable - many bacteria have at a certain stage of development, the tendency to become soft and ~~slightly~~ pleomorphic. They may at that stage, either in parts (?) or wholy, fuse with their neighbours or neighbour. Aggregates that do this may desintegrate (or as most people would say, become autolysed) or they may produce L-bodies and live on as such and even produce bacteria. If a pure culture is used, nothing more seems to happen. Yet when two "mating types" are mixed and grow together and by good fortune are in the same stage simultaneously , they produce in this way bodies of a more regular size which develop into zygotes. The zygote is a solid, deeply staining,

roundish, bacterial element of well defined contours which divides up regularly, and probably in two division steps reproduces bacteria. Or put it the other way round: zygote formation seems to be the purpose but when there is only one organism (one "mating type") then an unsuccessful attempt is made which (if not abortive altogether) results in the formation of L - bodies, which if suitably pampered, can grow in what I have called the L phase. Now the evidence I have collected so far :

At first I produced plates according to your instructions As inoculum I used washed mixtures of 58/161 and W11/77 or mixtures of 58/161 and W6/77 which Dr. Hayes had prepared. I found out that after about 14 hours of incubation I could regularly find groups of round bodies in twos, fours, and eights. I have already sent you the photos of these bodies from the plates which later grew prototroph colonies. After some experience I recognised these bodies easily, yet I had always to search for them quite considerably in my preparations. Let us call these very regular, deeply staining bodies,"zygotes". I now wanted to know how they develop. I found first that at edges of plates not quite inoculated to the margin I sometimes obtained more of my bodies. I have sent you photos of some lucky spots with many " zygotes". After this experience I started to produce microcultures, for the plates inoculated according to instruction are very unsuitable for finer observations. At first I did the microcultures in the following way: I took a sterile coverslip and placed a tiny drop of washed suspension (less thick than for the plates) in the middle. Then I placed a square of minimal agar (slightly smaller than the

coverslip) on top. They were incubated in a moist chamber. The coverslips (after removal of agar) are fixed (Osmic acid vapours) and stained (Giemsa) after desired periods of time. The "microscopical growth" of these cultures was quite good. Yet I found that if the cultures were not washed at all (of course, in disregard of the instruction of the genetecist!) I obtained much better results. (But I am no more out for prototrophs). All the photographs I am sending you today are from the edges of microcultures inoculated with unwashed suspensions, or rather, mixed young broth cultures.

The time required for the production of zygotes is longer than on the original plates. If the two cultures are investigated singly it can be seen that they develop cells of different appearence. W11/77 and W6/77 grow in short rods, 58/161 grows in long rods. After about a day or more W11/77 and W6/77 show areas where the small cells become very fady, they flow together and their attempt to produce "zygotes" or " L bodies" is perfectly abortive. 58/161 behaves much differently. It grows into long, deeply red staining filaments which become pleomorphic, fuse and produce L bodies, but I have never seen zygotes appear so far. When the cultures W11/77 or W6/77 and 58/161 are mixed, it is found that after a day or so of incubation the edge has the appearance shown in photos 12/52 and 13/52. The short rods are the elements of the W strain, the long ones are the elements of the 58 strain. After a further period of incubation the picture may be as in 14/52. The short rods are now pale blue and soft, the long ones deep

red. At this stage 58 becomes pleomorphic and fusions begin to occur. This is illustrated in 18/52. Here many of the pale blue cells have already faded away. Where fusion has occurred, bodies now begin to develop. Perhaps 20/52 is representative for this stage. There is still a lot of the pale blue material present. 10/52, 15/52, 16/52, 19/52, show similar pictures.

Sometime after this you may find a place as shown in 21/52 where I would suggest the round, darkly staining cells represent zygotes Many of them do not further develop, probably because they are lacking in growth factors. 11/52 A shows a very good group of zygotes which I think have divided up. Yet here I do no more get on very well and I propose now to feed secondarily the edges of my preps, with the various amino acids (growth factors) they may require. Perhaps you can here suggest something. I have not yet found out how, in the microcultures the zygotes reproduce bacteria. Anyhow, the whole story is not yet watertight and I feel I have to do a lot more work. Often also I miss the zygotes altogether in my preparations. They do not always develop at the same time, but I find them again and again and only in the mixed cultures.

One more photo, 6/52A . It is a pure 58/161 culture which has developed L bodies yet no zygotes. I hope that this very badly written account may give you an idea of what I am doing and what I am after. Perhaps you can make further suggestions.

Dr. Hayes read a paper at Oxford yesterday which I heard, I think the theory which he constructed of the gene donator and gene receptor strain, hangs a bit in the air and I do not like it very much.

I have not heard from Rockefeller's yet, but Dr.Pomerath came to see me not long ago.

Sincerely yours,

E. Nobel.

P. S.

I cannot send you yet, preserved Canada Balsam preparations, but maybe in some time. I should like to point out that the first photos I sent you sometime ago, represent W11/77 cells only (they developed from a mixed inoculum) which were in the stage of fusion; yet as there was no partner in these spots, these developments are abortive.

Of course the time factor is very important in the mating experiments. One organism may be ready in certain areas at a specific time, the other may not. Yet in other areas they may both be ready at a later time, for the cultures go on developing on the minimal agar for quite a time and one generation follows another. When strains are treated in certain ways this may affect the time factor, and no very complicated explanation is necessary.

Another point: When the two mating types are well mixed and both organisms are in the "fusion stage", it may well be that the fusions occurring are of three types (1) W strains with W cells, (2) 58 cells with 58, (3) W cells with 58 cells. Only the last fusions would produce real matings and zygotes. (1) would be abortive, (2) would produce L forms. It is not possible to see exactly what happens. Yet the pictures suggest to me that fusion between the pale blue and long red cells does occur.

E. N.